Republic of Kenya

EDICT OF GOVERNMENT

KS 03-929 (2009) (English): Specification for QAC based aromatic disinfectant liquid (Draft Standard)

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KENYA STANDARD

KS 03-929: 2009
ICS 67.160.20

Specification for QAC based aromatic disinfectant liquid

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Consumer Information Network
Kel Chemicals
University Of Nairobi
KIRDI
Jet Chemicals
Reckitt Benkiser
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ISBN 9966-23-619-8

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Specification for QAC based aromatic disinfectant liquid

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P R E F A C E

This Kenya Standard was prepared by the Technical Committee on Disinfectants, under the guidance of the Chemical Industry Standards Committee, and it is in accordance with the procedures of the Bureau.

QAC based aromatic disinfectant fluids are aromatic disinfectant fluids containing quaternary ammonium compounds as their principle antmicrobial agents. The quaternary ammonium compounds are water soluble. Disinfectant fluids are used for the destruction of micro-organisms, but not usually bacterial spores. They do not necessarily kill micro-organisms, but reduce them to levels, which are not harmful to health or the quality of perishable goods.

This standard covers five grades of QAC based aromatic disinfectant fluids which are designated according to their antimicrobial activity. This characteristics of disinfectants are very important to the user, thus the need for a standard. The test method included in this standard, counts the, number of viable organisms before and after the disinfecting process has been completed. In addition to antimicrobial value, the standard also specifies general composition, pH, stability and staining.

These QAC based aromatic disinfectants are used in households and domestic service disinfecting.

During the preparation of this standard, reference was made to the following documents.

BS 6424: 1984 Specification for QAC based aromatic disinfectant fluids.

BS 6471: 1984 Method for determination of the antimicrobial value of QAC disinfectant formulation.

NS 5197: 1976 Specification for aromatic disinfectant fluids.

The assistance obtained from the above documents is acknowledged with thanks.

KENYA STANDARD

SPECIFICATION FOR QAC BASED AROMATIC DISINFECTANT LIQUID

1. SCOPE

This Kenya Standard specifies requirements for five grades of light-duty, aromatic disinfectant fluids containing quaternary ammonium compounds (QACs) as their principal antimicrobial agents. The five grades are designated according to their antimicrobial activity, as measured by the method described in Appendix A.

2. APPLICATION

This standard applies to disinfectants used in households and public institutions such as schools, hotels and hospitals.

3. GRADE DESIGNATION

For a fluid to be designated as one of the following grades, it shall have an antimicrobial value, when determined by the method described in Appendix A not less than the value given below for the grades concerned.

Grade	*Minimum Antimicrobial Value*
QAP 30	30
QAP 50	50
QAP 100	100
QAP 200	200
QAP 300	300

4. REQUIREMENTS

4.1 General Composition — The fluids shall either be clear solutions or homogeneous suspensions containing one or more QACs which largely confer the disinfectant properties. They shall contain pine oil, related terpenes or other compatible odour-enhancing compounds.

They may also contain non-ionic surfactants or other builders and solubilizers, which, if used, shall not affect adversely either the activity of the disinfectant or the packaging.

4.2 Stability Before Dilution

4.2.1 *Normal* Storage *Stability* — After two separate samples have been stored for 3 months in accordance with the method described in Appendix B, at 10 ± 2°C and 35 ± 2℃ respectively, neither shall show any precipitate of solid matter or any separation into layers, and there shall be not a decrease in antimicrobial value to below the minimum specified in **3** for the grade concerned.

4.2.2 *Cold Storage Stability* — The fluids shall remain homogeneous after standing at 0℃ to 1℃ for 6 h in accordance with the method described in Appendix B.

4.2.3 *Hot Storage Stability* — The fluids shall remain homogeneous after standing at 40℃ for 6 h in accordance with the method described in Appendix B.

4.3 **Stability after Dilution** — Solutions of fluids in the standard hard water prepared shall be homogeneous at all dilutions down to that corresponding to twice the minimum antimicrobial value specified in **3,** for the grade concerned, and there shall be not any separation into layers after two separate such dilutions have been left standing for 6 h at 10 ± 2℃ and 35 ± 2℃, respectively.

Standard hard water is prepared by dissolving 0.304 g of anhydrous calcium chloride ($CaCl_2$) and 0.139 magnesium chloride hexahydrate ($MgC1_2.6H_20$) in water and dilution water to the mark in a 1006 mL one-mark volumetric flask. The hardness corresponds to 342 mg/L of hardness, calculated as $CaCO_3$.

4.4 **Staining** — If the fluid is recommended for use on fabrics, then the change in the whiteness value of the test fabric when tested according to Appendix C, shall not exceed 7 per cent of the whiteness value of the control sample.

4.5 **Packaging** — The fluids shall be packed in containers so that they can be stored at both 10 ± 2℃ and at 35 ± 2℃ for 12 months without any interaction b etween the container and the fluid that will bring the later outside the above requirement.

4.6 **Marking**—Each container of fluid or a label firmly attached to each container shall be marked legibly and indelibly with the following information:

(i) Name and address of manufacturer /distributor and registered trade mark, if any;

(ii) Batch or code number;

(iii) Net volume of contents;

(iv) The words QAC aromatic disinfectant giving its QAP value and active ingredient used.

(v) Date of manufacture and expiry date;

(vi) Recommendations for use in fabrics, if appropriate

(vii) Country of origin. Instructions for use, given in the imperative tense, including at least the dilutions recommended for particular applications and that the dilutions should be freely and accurately prepared in clean containers, together with the following:

(a) Either an indication that the main use of the fluid is to disinfect articles that are visibly clean or only lightly soiled.

(b) That heavily-soiled articles should be rinsed clean before being disinfected.

(c)A statement that no recommendation is made for use for disinfecting purposes at dilutions greater than that corresponding to the antimicrobial value.

(d)An instruction not to mix with soaps, anionic surfactants, acids and organic matter.

(e) Both date of manufacture and date of expiry together with the batch number.

(f) ‘Keep away from children’.

(g) 'ONLY FOR EXTERNAL USE' should be clearly labelled in capital letters together with ‘In case of accidental ingestion, take milk and seek medical attention immediately’.

A P P E N D I X A

DETERMINATION OF THE ANTIMICROBIAL VALUE OF QAC DISINFECTANT FORMULATIONS

A1. PRINCIPLE OF THE METHOD

A challenge medium is added to a series of dilutions of a product and, after a contact period of 600 ± 5 s at 22 ± 0.5℃, the mixture de-activated. Serial tenfold dilutions of the de-activated mixture are then incubated on pour plates a fixed time and the colonies counted. The procedure is repeated, as necessary until two dilutions differing in concentration by a ratio of 2:3 are obtained, one of which returns a colony count not greater than 0.01 per cent (i.e., 99.99 per cent 'Kill') of that returned by a control not containing the product and the other of which returns a colony count greater than 0.01 per cent of that returned by a control not containing the product.

A2. STERILIZATION

Wherever sterile reagents, media, material or apparatus are referred to, or an instruction to sterilize them appears, the sterility shall be achieved by being kept at either:

(i) 170℃ to 175℃ for not less than 1 h in an oven (dry sterilization); or

(ii) 121 ± 1℃ for 15 min in an autoclave (wet sterilization).

Manipulation of sterile material and bacterial cultures shall be carried out aseptically.

A3. REAGENTS AND MATERIALS

A3.1 **General** — All reagents shall be of recognized biological or analytical grade and distilled water. All solutions and media shall be freshly prepared.

A3.2 **Diluent** — Dissolve 10.0 g of calcium chloride hexahydrate ($CaCI_2.6H_20$) in water and dilute to 100 mL. Dissolve 10.0g of Magnesium sulphate heptalydrate ($MgSO_2.7H_2O$) in water and dilute to 100 mL. Add 3.5 mL of the calcium chloride solution and 1.0 mL of the magnesium sulphate solution to approximately 500 mL of water in 1L volumetric flask and dilute to the mark with water. This diluent corresponds to a synthetic hard water of 200 ± 10 mg/kg hardness.

A3.3 **Test Organism** — *Escherichia coli,* ATCC 11229.

A3.4 **Solid Culture Medium** — Oxoid nutrient agar CM3 sterilized in accordance with **A2.**

A3.5 **Liquid Culture Medium** — Standard strength, oxoid nutrient broth No. 2 sterilized in accordance with **A2.**

A3.6 **Inactivator** — Add 2 g of Soya lecithin and 3 g of between 80 to 95 mL of water, warm to 50℃ to 60℃ and stir until no visible lumps remain and the suspension is evenly opalescent. Dispense into capped containers in 9 mL quantities and sterilize in accordance with **A2.**

A3.7 **Ringer's Solution** — **Quarter-strength** — Dissolve 9.00 g of sodium chloride, 0.42 g of potassium chloride, 0.24 g of anhydrous calcium chloride and 0.20 g of sodium hydrogen carbonate in water and dilute to 100 mL.

Add 1 volume of this solution to 3 volumes of water to give a quarter-strength solution. Dispense into 150/16 mm test tubes fitted with suitable colours and sterilize in accordance with **A2.** Ringer's solution tablets may also be used. Dissolve 1 tablet in the appropriate volume of water to obtain a quarter-strength solution, fill 150/16 mm test tubes fitted with suitable closures and sterilize according to **A2.**

A3.8 Horse Serum Oxide SR 35

A3.9 Check disinfectant, clodecyldimethyl-s-phenoxyethyl ammonium bromide, 75 mg/L and 125 mg/L solutions.

A4. APPARATUS

A4.1 Ordinary microbiological laboratory apparatus especially as given below:

A4.1.1 *2 mL pipettes*

A4.1.2 *Universal container culture bottles,* 28 mL capacity

A4.1.3 *Petri dishes*

A4.1.4 *Water bath*, *capable of being controlled at 22 ± 1 ℃.*

A4.2 Cleanliness — Special care in the use and cleaning of glassware is necessary because of the strongly absorptive properties of QAC. The following cleaning procedure should be followed:

Wash glassware in cold 62 g/L nitric acid solutions and allow to stand overnight in the acid. Rinse successively with 4 g/L sodium hydroxide solution, tap water, 6 g/L nitric acid solution and tap water until the acid is removed. Finally, rinse with distilled water.

A5. PREPARATION OF TEST CULTURE

A5.1 Initial Culture — The test organism (**A3.3**) is distributed in tubes in freeze-dried form, and shall be reconstituted in accordance with the supplier's instructions.

Using a sterilized pipette, add approximately 0.5 mL of the sterilized liquid culture medium (**A3.5**) to the contents of the tube and incubate for 25 hours at 37 ± 1℃. From this initial culture, prepare the sto ck culture (**A5.2**) and, from that, the broth cultures (**A5.3**)**.**

A5.2 Stock Culture — Spread a loopful of the initial culture over the surface of a 'slope' of the sterilized solid culture medium (**A3.4**)**.**

Incubate for 24 h at 37 ± 1℃ and store at not abov e 22℃, and preferably between 4℃ and 10℃, until required. Stock cultures so stored should be used within 1 month, although sub-cultures can be taken before the expiry of that date. Not more than six serial sub-cultures shall, however, be taken before resorting to a new freeze-dried tube (**A3.3**)**.**

A5.3 Broth Culture — Inoculate a tube of 10 mL of the liquid culture medium (**A3.5**) from the stock culture, (**A5.2**) and incubate at 37 ± 1℃ for 22 h to 26 h. Progres sively sub-culture into fresh liquid culture medium every 24 h, incubating the inoculum at 37 ± 1℃ for 22 h to 26 h and use sub-culture 2 to 6 fo r the preparation of the challenge medium (**A6**). After six broth-to-broth sub-cultures, restart the process using fresh stock culture.

A6. PREPARATION OF CHALLENGE MEDIUM

From the broth culture (**A5.3**) prepare aseptically a challenge medium according to the following formula:

(i) broth culture (**A5.3**): 2 paits by volume.

(ii) horse serum (**A3.8**): 5 paits by volume.

(iii) diluent (**A3.2**): 3 paits by volume.

Hold at 22℃ for not more than 4 h. The challenge medium so prepared should contain between 5 x 10^7 and 5 x 10^8 colony-forming units per millilitre, measured as described in **A8**.

A7. PREPARATION OF DILUTIONS

A7.1 General — Dilutions of the product shall be prepared volumetrically by pipette and sufficiently over-strength to allow the further dilution when the 1 mL of challenge medium is added. For example, prepare a 1:100 dilution at 1:90.

Prepare all dilutions in the diluent (**A3.2**) and include two containers of 9 mL of the diluent alone in each series carried out at the same time, to function as controls.

Place 9 mL of each dilution in sterile universal container culture bottles and hold in a waterbath at 22 ± 0.5℃ until equilibrated at that temperatur e.

All dilutions shall be prepared and tested in the same working day.

A7.2 Range of Dilutions

A7.2.1 If t he potency of the product is known approximately, prepare three dilutions of concentration 3 x /2, x, 2 x /3, respectively, where x is the concentration that is expected to return a colony count of about 0.01 per cent of that returned by the control. If a satisfactory result is not obtained using this choice of dilutions, proceed to a more appropriate choice.

A7.2.2 If the product has a completely unknown potency, carry out a preliminary test on a series of widely-ranged dilutions, such as 1:5, 1:25 and 1:125, and repeat the test on successively narrow ranges until the aim of the test is achieved.

A7.2.3 If the potency is known only in terms of QAC content, carry out a preliminary test on dilutions equivalent to 300 mg/L, 200 mg/L and 100 mg/L, and then repeat the procedure as described in **A7.2.2** until the aim of the test is achieved.

A8. TEST PROCEDURE

A8.1 At time 0, pipette 1 mL of the challenge medium (**A6**) into 9 mL of each of the dilutions (**A7.1**) under test in both of the 9 mL portions of the control diluent (**A7.1**) and mix by gentle rotation. Ensure that every trace of challenge medium is brought into full contact with the disinfectant but avoid foam formation. Maintain at 22 ± 0.5℃ until the end of the contact period of 600 ± 0.5 s and shortly before the end of this contact period, repeat the mixing.

A8.2 At time 0 + (600 ± 5) s, deposit 1 mL of the mixture into 9 ml of the inactivator and mix the contents by shaking.

A8.3 Within 1 h of inactivation, make serial decimal dilutions in the Ringer's solution (**A3.7**).,Prepare delicate pour plates from 1 mL of these dilutions and 10 mL of the sterilized solid culture medium (**A3.4**) after the latter has been melted and equilibrated at 45℃. Al low to set, invert the plates and incubate at 37 ± 1 ℃ in the inverted position for 48 ± 2 h and then count the colonies.

A9. TEST RESULT

After appropriate calculation, record the antimicrobial value as the greatest dilution of the product, in the volume by volume terms (or in mass by volume terms in the case of solid or very viscous products), that returns a colony count not greater than 0.01 per cent average of the colony count returned by the two diluent controls.

For example, if the greatest such dilution is 1:100, the antimicrobial value is 100.

If there has been a significant reduction in the number of viable cells in the controls during the test period, repeat the test

A P P E N D I X B

METHOD OF TEST FOR STABILITY BEFORE DILUTION

Store the samples in round, narrow necked, clear bottles of 250 mL to 300 mL capacity, fitted with screw caps with ment liners. Ensure that the ullage does not exceed 5 per cent and that the samples are protected from direct sunlight and from localized overheating and overcooling during the storage period.

A P P E N D I X C

STAINING TEST FOR AROMATIC DISINFECTANT FLUIDS

C1. MATERIALS

C1.1 Test Fabrics — The fabric for the test shall be bleached cotton lawn free from filling and from fluorescent brightening agents. The fluidity shall be not greater than 4.00. The weight shall be 94 gm^2.

Six pieces of fabric, 30 cm x 15 cm, are required, three for each test with the disinfectant fluids and three to serve as controls.

C1.2 Disinfectant Dilutions — Use a dilution factor equal to one in 10 times the claimed Rideal-Walker coefficient or antimiziobial value in this case. For dilution use distilled water.

C2. PROCEDURE

C2.1 Treatment of Test Pieces — Form each piece of a test fabric into a loose coil that can be suspended vertically in a beaker or similar vessel. Using a separate portion of the diluted fluid for each piece of fabric, immerse the three pieces in the diluted disinfectant fluid and leave at room temperature for 6 h.

At the end of 6 h squeeze each test piece by hand and transfer it to 500 mL of water (distilled water). Using a glass rod, stir the piece gently for 1 min, then discard the water and repeat the rinsing in a further 500 mL of water. Remove the piece from the water, squeeze it by hand and hang it lengthwise in air to dry.

Do not allow the pieces to become so dry that they are difficult to iron flat.

Iron the test pieces, preferably with a steam iron, but otherwise with a domestic electric iron fitted with a thermostatic control. Set the control just below t a setting for cotton. Remove all creases from the test pieces.

C2.2 Control Pieces — Treat the three control pieces in exactly the same manner as the test pieces but use distilled water instead of the diluted disinfectant fluid.

C2.3 Visual Assessment of Staining — Place the test pieces and the controls in separate piles, folded to give six thicknesses of fabric. Examine the two pieces side by side, in a good natural light and away from coloured reflecting surfaces, and note whether or not there is a visible difference in colour. Examine both sides of each piece of fabric.

C2.4 Measurement of Staining (for Reference Purposes) — Place the three test pieces, folded to give a total of six thicknesses of fabric, in a single pile, and measure the reflective value by means of a photoelectric reflectometer, using white light. Make four measurements on each side of each of the three test pieces. Repeat this procedure with the control pieces. For each set of pieces, calculate the mean of all the readings and record this as the whiteness value.

C3. CALCULATION OF RESULT (REFEREE METHOD)

Calculate the loss of whiteness due to the disinfectant by means of the following formula:

Loss of whiteness (1 per cent) $\frac{R_1 - R_2}{R_1} \times 100$

where,

R_1 = the whiteness value for control pieces,

R_2 = the whiteness value for fabric treated with disinfectant.